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EXAMINER	
SKELDING, ZACHARY S	
ART UNIT	PAPER NUMBER
1644	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/676,045	ILAN ET AL.	
	Examiner	Art Unit	
	ZACHARY SKELDING	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *3-10-08 4-17-08*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3,5-13,15-20,23-46,50-63,66-72,83-126 and 143-166* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *2,3,6-13,15,19,24,30-32,144-151,165 and 166* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *05 March 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1-26-04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims withdrawn from consideration are 1,5,16-18,20,23,25-29,33-46,50-63,66-72,83-126,143 and 152-164.

DETAILED ACTION

1. Applicant's election, with traverse, in the responses filed and March 10, 2008 and April 17, 2008 are acknowledged.

   Claims 4, 14, 21, 22, 47-49, 64, 65, 73-82 and 127-142 have been canceled.

   Claims 1-3, 5-13, 15-20, 23-46, 50-63, 66-72, 83-126 and 143-166 are pending.

2. Applicant's election with traverse of Group II and various species of invention in the reply filed on April 17, 2008 is acknowledged.

   The traversal is on the ground(s) that "Groups I, II, IX and X all encompass claims that 1) seek to modulate the Thl/Th2 cell balance; 2) manipulate the NKT cell population in some way to modulate the Thl/Th2 cell balance; and 3) treat an immune- related or immune-mediated disorder or disease in a mammalian subject." Applicant makes similar arguments about Groups V and VIII.

   Applicant's arguments are not found persuasive because (1) the inventions as claimed are not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.05(j). For example, the inventions are mutually exclusive in that the same method steps can't be used to both promote and decrease inflammation. Moreover the claimed methods have diametrically opposing endpoints proinflammatory versus anti-inflammatory. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

   Applicant further argues the inventions of V/VI and II/IX, respectively, which are related as product and process of use are not patentably distinct because treatment with corticosteroid is general suppression rather than NKT cell specific suppression.

   Applicant's argument is not found convincing because applicant has not convincingly argued why a generalized corticosteroids mediated immunosuppression is not encompassed by the breadth of the claimed invention. Moreover, it is noted that the claimed method can be practiced by administering other materially different products, e.g., α-Galcer.

   Applicant argues the inventions of VIII/VI and I/X, respectively, which are related as product and process of use are not patentably distinct because treatment with an adjuvant is a general inflammatory booster rather than NKT cell specific booster.

   Applicant's argument is not found convincing because applicant has not convincingly argued why a generalized adjuvant mediated inflammation is not encompassed by the breadth of the

claimed invention. Moreover, it is noted that the claimed method can be practiced by administering other materially different products, e.g., IL-12 and anti-CD3 antibody.

Regarding the species election for the specific immune-related or immune-mediated disorder to be treated, Applicant traverses on the ground that (emphasis added), "*the species are not patentably distinct. All of these diseases or disorders are obvious variants of each other - they can all be treated by shifting the Th1/Th2 cell balance towards Th2, an anti-inflammatory response*. Furthermore, treatment of all of these disorders or disease results in an increase of the CD4+ IL4+IL10+/CD4+ IFNγ ratio."

Applicant's clear admission that the various species of immune-related or immune-mediated disorders to be treated are "obvious variants of each other" is duly noted.

While applicant may very well have made a valid assertion for some of the diseases recited in the claims encompassed within elected Group II, the examiner submits that applicant's assertion is not true for all of the diseases encompassed within elected Group II.

For example, an overabundance of NKT cells expressing high IL4/IFNγ is found in the lungs of patients suffering from a lung disease, in particular asthma, or in patients suffering from autoimmune liver disease (see below), and in both diseases these high IL4/IFNγ cells are thought to directly cause disease pathogenesis. Thus, it is unclear how all of the diseases encompassed within elected Group II are obvious variants of each other in that some of the diseases recited in the claims encompassed by Group II cannot be treated by modulation of NKT cells to increase their IL4/IFNγ ratio.

Applicant further argues the various culture conditions species encompassed within the claims of elected Group II are not patentably distinct because all the recited antigens or epitopes are associated with the development of particular disorders or diseases, because the liver associated cells all come from the liver and because the recited cytokines are all biologically active molecules.

Applicant's argument is not found convincing, essentially for the reasons of record as put forth in the Restriction Requirement mailed September 11, 2007.

For example, as explained further in the scope of enablement rejection put forth below some of the cytokines encompassed by the elected claims would not increase the IL4 IL10/IFNγ ratio.

The requirement is still deemed proper and is therefore made FINAL.

However, upon further consideration, the "Crohn's disease" species of "immune-related or immune-mediated disorders to be treated" has been rejoined.

Thus, claims 2, 3, 6-13, 15, 19, 24, 30-32, 144-151, 165 and 166 are under consideration as they read on a method for the treatment of immune-related or immune-mediated disorders or diseases in a mammalian subject in need of such treatment, by manipulating the NKT cell population of said subject, wherein manipulation of said NKT cell population results in modulation of the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, said modulation being mediated by different components, cells, tissues or organs of said subject's or another subject's immune system comprising the steps of:

a. obtaining NKT cells from said subject or another subject;

b. *ex vivo* educating the NKT cells obtained in step (a) such that the resulting educated NKT cells may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells; and

c. re-introducing to said subject the educated NKT cells obtained in step (b) which may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ratio between any one of IL4 and IL 10 to IFNγ,

wherein said *ex vivo* education of step (b) is performed by culturing said NKT cells in the presence of any one of:

a. antigens or epitopes associated with said immune-related or immune-mediated disorder or disease to be treated, antigens or epitopes associated with the immune-mediated inflammatory response, or any combination thereof;

b. at least one liver-associated cell of tolerized or non-tolerized subjects suffering from said immune-related or immune-mediated disorder or of said subject;

c. at least one cytokine or adhesion molecule, or any combination thereof; and

d. a combination of any of (a), (b) and (c), wherein the species of "immune-related or immune-mediated disorders or diseases" is "autoimmune liver disease" or "Crohn's disease"; the species of "culture conditions for the ex vivo education of NKT" includes "allogenic antigens obtained from donors suffering from said immune-related or immune-mediated disease", "Kupffer cells" and "IL4".

Accordingly, claims 1, 5, 16-18, 20, 23, 25-29, 33-46, 50-63, 66-72, 83-126, 143, 152-164 have been withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected Group and/or species of invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on March 10, 2008.

3. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 2, 3, 6-13, 15, 19, 24, 30-32, 144-151, 165 and 166 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for

a method for the treatment of TNBS-induced-colitis in a first mouse in need of such treatment comprising:

(1) orally administering to said first mouse colitis extracted proteins (CEP) prepared from colons that were removed from TNBS-induced-colitis mice, cut into small strips, mechanically homogenized, filtrated through a 40 mm nylon cell strainer, and the colitis extract supernatant isolated from intact cells via centrifugation;

(2) obtaining 0.5 x 10^6 liver associated lymphocytes and 2.5 x 10^6 splenocytes from a second mouse that had been treated with TNBS to induce colitis and had been orally administered CEP prepared as in step (1);

(3) adding to a culture of the 0.5 x 10^6 liver associated lymphocytes and 2.5 x 10^6 splenocytes from step (2) antigen presenting cells and CEP prepared as in step (1);

(4) optionally adding to said culture IL4, IL10, TGFβ, IL18 or IL15,

(5) administering the cultured cells of step (3) to the first mouse in need of such treatment to modulate the Th1/Th2 balance toward anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ratio between any one of IL4 and IL10 to IFNγ

does not reasonably provide enablement for

a method for the treatment of immune-related or immune-mediated disorders or diseases in a mammalian subject in need of such treatment, by manipulating the NKT cell population of said subject, wherein manipulation of said NKT cell population results in modulation of the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, said modulation being mediated by different components, cells, tissues or organs of said subject's or another subject's immune system comprising the steps of:

a. obtaining NKT cells from said subject or another subject;

b. *ex vivo* educating the NKT cells obtained in step (a) such that the resulting educated NKT cells may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells; and

c. re-introducing to said subject the educated NKT cells obtained in step (b) which

may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ratio between any one of IL4 and IL 10 to IFNγ,

wherein said *ex vivo* education of step (b) is performed by culturing said NKT cells in the presence of any one of:

a. antigens or epitopes associated with said immune-related or immune-mediated disorder or disease to be treated, antigens or epitopes associated with the immune-mediated inflammatory response, or any combination thereof;

b. at least one liver-associated cell of tolerized or non-tolerized subjects suffering from said immune-related or immune-mediated disorder or of said subject;

c. at least one cytokine or adhesion molecule, or any combination thereof; and

d. a combination of any of (a), (b) and (c),

and wherein said NKT cells may optionally express the CD56 marker.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in In re Wands, 8 USPQ2d 1400 (CA FC 1988). Wands states on page 1404, "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The instant specification discloses a number of working examples some of which are highlighted below:

Example 1 teaches how "colitis extracted protein" (CEP) was isolated, the method used for NK1.1 cell depletion with anti-NK1.1 antibody, and the method used to prepare splenocytes and liver associated lymphocytes (see instant specification page 61, 3rd and 4th paragraphs and page 64, 1st paragraph, respectively).

Examples 4-5 (instant specification pages 74-79) teaches that TNBS mice fed CEP during the 14 day period following administration of TNBS on day 1 ("Group J") suffered far less colitis symptoms than, for example, mice treated in the same way but fed BSA ("Group H").

Moreover, mice which were fed CEP during the 14 day period following administration of TNBS on day 1, but exposed to an anti-NKT1.1 antibody that depletes the majority of NKT1.1+ cells from their liver, spleen, bone marrow and peripheral blood just 36 hours prior to termination, i.e., on day 12.5 ("Group I"), the colitis observed in these mice was similar to the mice fed BSA.

Moreover, after termination $1x10^6$ splenocytes were isolated from all of these differently treated mice and transferred to recipient mice who had been sublethally irradiated with 300 rad total body irradiation. 24 hours after injection of the splenocytes the recipient mice were treated with TNBS and then terminated 13 days later. The recipient mice receiving splenocytes from the mice fed CEP during the 14 day period exhibited very little colitis ("Group J' "). The recipient mice receiving splenocytes from mice fed BSA had severe colitis ("Group H' "), and the recipient mice that had received splenocytes obtained from mice fed CEP but in which NK1.1+ cells were depleted at day 12.5 exhibited the worst colitis of all ("Group I' ").

Moreover, as was seen with the donor mice, the splenic lymphocytes isolated from the recipient mice after termination exhibited a high IL4/IFNγ ratio in mice that received the splenocytes from Group J but mice receiving splenocytes from Groups I and H had an around 4 fold smaller IL4/IFNγ ratio.

Example 6 (instant specification pages 79-80) teaches that LAL isolated after terminating the recipient mice receiving splenocytes from the mice fed CEP during the 14 day period which exhibited very little colitis ("Group J' ") demonstrated that these lymphocytes were comparatively more cytotoxic against YAC-1 target cells than, for example, LAL isolated after terminating the recipient mice receiving splenocytes from mice fed BSA which had severe colitis ("Group H' "), and the recipient mice that had received splenocytes obtained from mice fed CEP but in which NK1.1+ cells were depleted at day 12.5 and which exhibited the worst colitis of all ("Group I' ") (see instant specification Figure 11).

Example 3 (instant specification pages 74-77) teaches the evaluation of the effect of *in vitro* exposure to the disease-target antigen on the CD4 + IL4 +/ CD4 + lFNy + ratio. As in example 4, TNBS treated mice were fed CEP over a 14 day period following administration of TNBS on day 1 ("Group B"), some mice were treated in the same way but fed BSA ("Group A") and yet other mice were fed CEP during the 14 day period following administration of TNBS on day 1, but exposed to an anti-NKT1.1 antibody that depletes the majority of NKT1.1+ cells from their liver, spleen, bone marrow and peripheral blood just 36 hours prior to termination, i.e., on day 12.5 ("Group E").

Upon termination, 0.5 x 10^6 liver associated lymphocytes (LAL) and 2.5 x 10^6 splenocytes were harvested from these mice and cultured for 12 hours in the presence of Con A and in the absence of CEP and APC or cultured for 72 hours in the presence of CEP and APC. As shown in Figure 5, for those animals that had been fed CEP and administered TNBS ("Group B"), upon exposure to antigen presenting cells and CEP in vitro LAL and splenocyte cells from these mice exhibited a substantial increase in IL-4/IFNγ ratio. Moreover, when animals were treated the same as Group B animals but NK1.1 cells were depleted 36 hours prior to termination ("Group E"), the IL-4/IFNγ ratio was substantially decreased and the difference between incubation with Con A versus incubation with CEP and APC was narrowed (compare white and black bars for Group E in Figure 5).

Furthermore, upon termination supernatant fluids were isolated from the 0.5 x 10^6 liver associated lymphocytes (LAL) and 2.5 x 10^6 splenocytes and tested for the amount of IL-4 and IFN-γ. As can be seen in Figure 6, animals that had been fed CEP and administered TNBS ("Group B") showed low levels of IL-4 and even lower IFNγ. In contrast, when animals were treated the same as Group B animals but NK1.1 cells were depleted 36 hours prior to termination ("Group E"), there was a substantial increase in the amount of IFNγ production without a substantial change in IL-4 levels.

Some other facts commonly known in the art are helpful to put the teachings of the instant specification into the context of the knowledge in the art. First, it is well known in the art that murine spleen constitutes around 3% NKT cells, around 33% conventional T cells, and around 33% B cells while murine liver has from 30-50% NKT cells (see, e.g., Godfrey et al., Immunol Today. 2000 Nov;21(11):573-83, in particular page 575, left column, 2nd paragraph).

Furthermore, based on the splenocyte preparation procedure disclosed in the instant specification, the skilled artisan would consider the isolated and purified spleen lymphocytes to contain about 80-90% conventional T cells/NKT cells and 10-20% B cells given that nylon screen purification is not too precise (see, e.g., Ada M. Kruisbeek, *Current Protocols in Immunology,* John Wiley & Sons (2000), 3.1.1-3.1.5 and Karen S. Hathcock, *Current Protocols in Immunology,* John Wiley & Sons (1992), unit 3.2.1-3.2.4.).

With respect to the LAL purification procedure disclosed in the instant specification, the skilled artisan would consider the isolated and purified LAL lymphocytes to contain a majority of NKT cells + conventional T cells and potentially some fraction of B cells given the presence of B cells in murine fetal liver and the imprecision of nylon screen purification (see ibid); however it is noted that very little was known about the number of B-cell in the murine adult liver as of applicant's date of invention (see, e.g., Novobrantseva et al., J Clin Invest. 2005 Nov;115(11):3072-82, in particular, page 3072, left column, 1st paragraph) so the skilled artisan would have substantial uncertainty about the B cell content of purified LAL lymphocytes.

Thus, from the disclosure of the instant specification the skilled artisan would understand that orally administered CEP, in and of itself, can be used to inhibit the development of colitis in TNBS mice. Moreover, given the ability of NK1.1 cell depletion 36 hours before termination to induce colitis even in a mouse fed CEP (see instant specification Examples 4-5), in conjunction with the effect of 36 hour NKT cell depletion on IFN-γ expression (see instant specification Figure 6, Groups B vs. E) in these same mice fed CEP, the skilled artisan would consider NK1.1 cells to be of great importance in mediating the beneficial effects of orally administered CEP by either directly or indirectly suppressing IFN-γ expression. Lastly, given the increase in the IL4/IFNγ ratio observed when 0.5 x 10^6 LAL and 2.5 x 10^6 splenocytes harvested from CEP fed and TNBS treated mice are cultured for 72 hours in the presence of CEP and APC, the skilled artisan would come to understand that culturing 0.5 x 10^6 LAL and 2.5 x 10^6 splenocytes isolated from a "tolerized" mouse with CEP and APC allows for an increase in IL4/IFNγ ratio that extends beyond that obtained via culturing said LAL and splenocytes in the presence of a T-cell mitogen such as Con A.

Given that all of the data from the instant specification outlined above seems to be identically disclosed in prior art reference WO 02051986 of Yaron Ilan, the knowledge in the art of treating TNBS-induced-colitis in a first mouse in need of such treatment comprising:
(1) orally administering to said first mouse colitis extracted proteins (CEP) prepared from colons that were removed from TNBS-induced-colitis mice, cut into small strips, mechanically homogenized, filtrated through a 40 mm nylon cell strainer, and the colitis extract supernatant isolated from intact cells via centrifugation;
(2) obtaining 0.5 x 10^6 liver associated lymphocytes and 2.5 x 10^6 splenocytes from a second mouse that had been treated with TNBS to induce colitis and had been orally administered CEP prepared as in step (1);
(3) adding to a culture of the 0.5 x 10^6 liver associated lymphocytes and 2.5 x 10^6 splenocytes from step (2) antigen presenting cells and CEP prepared as in step (1);
(4) optionally adding to said culture IL4, IL10, TGFβ, IL18 or IL15
(5) administering the cultured cells of step (3) to the first mouse in need of such treatment to modulate the Th1/Th2 balance toward anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ratio between any one of IL4 and IL10 to IFNγ,
was high.

However, what the skilled artisan would not know from the disclosure of the instant specification is how to practice to its full breadth a method for the treatment of immune-related or immune-mediated disorders or diseases in a mammalian subject in need of such treatment, by manipulating the NKT cell population of said subject, wherein manipulation of said NKT cell population results in modulation of the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, said modulation being mediated by different components, cells, tissues or organs of said subject's or another subject's immune system comprising the steps of:
a. obtaining NKT cells from said subject or another subject;
b. *ex vivo* educating the NKT cells obtained in step (a) such that the resulting educated NKT cells may modulate the Th1/Th2 cell balance toward anti-inflammatory

cytokine producing cells; and
c. re-introducing to said subject the educated NKT cells obtained in step (b) which may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ratio between any one of IL4 and IL 10 to IFNγ,
wherein said *ex vivo* education of step (b) is performed by culturing said NKT cells in the presence of any one of:
a. antigens or epitopes associated with said immune-related or immune-mediated disorder or disease to be treated, antigens or epitopes associated with the immune-mediated inflammatory response, or any combination thereof;
b. at least one liver-associated cell of tolerized or non-tolerized subjects suffering from said immune-related or immune-mediated disorder or of said subject;
c. at least one cytokine or adhesion molecule, or any combination thereof; and
d. a combination of any of (a), (b) and (c),
and wherein said NKT cells may optionally express the CD56 marker.

First, the instant claims, given their broadest reasonable interpretation consistent with the instant specification read on a method of treatment comprising ex vivo education of NKT cells wherein the only cell present is an NKT cell.

The instant specification provides a single example of obtaining NKT cells, in particular, CD3+ NK1.1+ NKT cells, from a mouse and *ex vivo* "educating" the NKT cells in the presence of colitis extracted proteins (CEP) obtained from a mouse with TNBS induced colitis, Example 7 on pages 79-85. In this example whether said NKT cells were isolated from a mouse treated with TNBS, or from a mouse treated with TNBS and orally administered colitis extract protein (CEP) obtained by treating mice with TNBS and then harvesting the colon and preparing a protein extract according to the methods put forth on page 32, 3rd paragraph, or ex vivo educated in the presence of CEP did not seem to have any obvious consistent effect on the IL-10 to IFN-γ ratio (See Table 6, pages 63-66, in particular, E''2, E''3, E''5 and E''6).

It should further be noted that while NKT cells make up just a small percentage of T cells found in the murine spleen (3% according to Godfrey et al., Immunol Today. 2000 Nov;21(11):573-83, see in particular, page 575, left column, 2nd paragraph, at least for animals not TNBS treated), and furthermore that among these splenic NKT cells, there are at least 3 and possibly 4 functionally distinct subsets, of which CD4+ NKT cells are but one, CD4+ T cells showed very similar IL-10/IFN-γ expression as NKT cells when tested in the same way (See Table 6, pages 63-66, in particular, A''2, A''3, A''5 and A''6).

Thus, from the data of the instant specification, it is unclear what, if any effect, ex vivo education in the presence of CEP has on the NKT T cells, the most consistent discernable factor seemingly if the NKT cells were obtained from the splenocytes of a mouse treated with TNBS or non-treated mouse, not if the cells were exposed to CEP ex vivo or not.

It is further noted that "an NKT cell" is not just one type of cell but a genus of cells with differing cell surface markers, differing antigen-presenting molecule restriction specificities, and in turn differing modes of activation and differing effector functions. (See, for example, Kronenberg et al., Nat Rev Immunol. 2002 Aug;2(8):557-68, especially page 557 to page 558, left column and Table 1).

For example, "classical" murine NKT cells express the invariant T cell receptor Vα14-Jα18/Vβ8.2, Vβ7 or Vβ2 (referred to as "Vα14i" NKT cells by Kronenberg). Murine Vα14i cells, via their invariant αβ T cell receptor, recognize an antigen bound to a β2-microglobulin-associated nonpolymorphic, nonclassical CD1d MHC molecule. As of applicant's date of invention, physiologically relevant, naturally auto- or allo- antigens recognized by murine Vα14i NKT cells were unknown (however a "naturally" occurring small molecule isolated from a marine sponge, α-galcer, was known in the art to be a CD1d binding molecule that is recognized by murine Vα14i NKT cells in the context of CD1d and potently activates said cells in the presence of appropriate costimulatory signals (see Kronenberg, Box 1 "invasion of the murine sponges?" and below).

Moreover, while the instant specification discloses how to use colitis extract proteins to induce "oral tolerance" in TNBS treated mice as in Example 4-5 or to enhance the IL4/IFNγ ratio when added to 0.5×10^6 liver associated lymphocytes (LAL) and 2.5×10^6 splenocytes harvested from TNBS treated mice fed CEP over a 14 day period as in Example 3, neither the instant specification nor the art seem to recognize what particular biomolecular constituent, such as a particular protein, nucleic acid or lipid or cellular constituent of CEP, such as adherent microbial cells or colon epithelial cells are sufficient to mediate its biological effects when administered orally or to LAL and splenocytes in the presence of APC (see, e.g., Lee et al., Am J Gastroenterol. 2000 Apr;95(4):861, left column, 3rd paragraph and Kiron Das et al., Am J Gastroenterol. 2006 Dec;101(12):2889-90 and Margalit et al., Am J Gastroenterol. 2006 Dec;101(12):2890-91, in particular page 2890, right column, 2nd paragraph).

Furthermore, the skilled artisan could not extrapolate with any reasonable degree of predictability from the disclosure of the instant specification to treating any immune-related or immune-mediated disorder as claimed since for many diseases it is unclear what antigens or epitopes associated with an immune-related or immune-mediated disorder, if any, would be capable of inducing NKT cells to modulate the Th1/Th2 balance.

This is particularly unpredictable in that even setting aside the issue of what antigens or epitopes associated with an immune-related or immune-mediated disorder, if any, could be used to induce NKT cells to modulate the Th1/Th2 balance, oral tolerance, per se, as method of treating immune diseases has not lived up to its expectations, even where the oral tolerogen was a verified autoantigen.

For example, see Pozzilli et al. (Diabetologia 2000, 43:1000-1004), which reports that while the induction of tolerance was expected, oral administration of insulin to type I diabetics

simply does not have a statistical effect on the insulin autoimmunity. See also Wiendl et al., BioDrugs. 2002;16(3):183-200, in particular pages 194-195 which describes how orally administered bovine myelin basic protein showed no significant medical effect in a consecutive multicenter, double-blind, placebo-controlled phase III study of 516 patients with relapsing-remitting MS. Lastly, see Margalit et al., Am J Gastroenterol. 2006 Dec;101(12):2890-91 referring to the "disappointing results" of previous clinical trials involving oral administration of autoantigens.

Thus, the skilled artisan has considerable unpredictability in effectively treating an autoimmune disorder comprising orally administering a known autoantigen, and in turn the unpredictability in treating any immune-related or immune-mediated disorder using the method as claimed is even greater in light of the uncertainty as to what antigens or epitopes associated with immune-related or immune-mediated disorders are capable of educating NKT cells to modulate the Th1/Th2 cell balance toward anti-inflammatory cytokines.

Furthermore even assuming, arguendo, that CD3+ NK1.1+ NKT cells could be obtained from a mouse and *ex vivo* "educated" in the presence of colitis extracted proteins (CEP) obtained from a mouse with TNBS induced colitis, the instant specification does not give sufficient direction or guidance to use any particular NKT cell in the claimed method because while most murine Vα14i NKT cells are also NK1.1+, other NK cells which have a different T cell repertoire (with greater diversity) also express NK1.1 in some cases *but* may or may not be CD1d restricted, and the antigen recognized by these cells has not yet been determined (see Kronenberg, Table 1, NKT cell categories II-IV).

However, it is worth emphasizing that even when considering only the murine Vα14i NKT cells themselves, Kronenberg at page 557, right column, 2nd paragraph teaches "the overlap between expression of NK1.1 and Vα14*i* is incomplete. The expression of NK1.1 depends on maturity, activation state and tissue location. It also depends on the expression of the C57BL/6 NK gene-complex haplotype, as well as the genetic background…Therefore, because many of these cells do not express NK1.1,we refer to the category-I population as Vα14i T cells, rather than NKT cells."

With respect to humans, the equivalent of murine Vα14i NKT cells are "Vα24i" NKT cells which are similar to the murine cells in that they have a very restricted TCR repertoire and in that they recognize marine sponge α-galcer bound to Cd1d.

However, the differences between the biology of human and mice NKT cells as a whole far exceed the similarities.

For example, Kronenberg notes that Vα24i NKT cells are less frequent in humans than Vα14i NKT cells in mice, and in humans Vα24i T cells can also be CD8+ unlike in mice (see Kronenberg page 557, right column, 1st paragraph).

With respect to the heterogeneity of murine and human Vα14i and Vα24i T cells and the uncertainty in the art of the in vivo effect of activating said cells, Kronenberg further teaches on page 564, left column, 1st paragraph, emphasis added, "Do all of these cells behave similarly, or are there subsets that have distinct cytokine profiles after activation? In humans, the $CD4^+$ Vα24*i* T cells produced more IL-4 than their DN counterparts, but were less able to induce the expression of perforin after exposure to cytokines. By contrast, when mouse liver Vα14*i* T cells were analyzed *ex vivo* after a brief *in vivo* stimulation with α-GalCer, we found that nearly all of the activated cells had intracellular IFN-γ and IL-4 (J. L.Matsuda, L.G., S. Sidobre and M.K., unpublished observations). *These differences might reflect differences in the organ and/or species that was analyzed, or in the experimental systems that were used.* A third unresolved issue concerns the effect of Vα14*i* T-cell activation on the ongoing or subsequent adaptive immune response. In some studies, immunization with α-GalCer on one or more occasions led to an overall TH2 response, with increased total serum levels of IgE and augmented IL-4 responses, by conventional T cells that were stimulated with peptide antigens. The opposite result - decreased serum IgE and an IFN-γ-fuelled TH1 response after α-GalCer treatment - has been reported also. *This discrepancy is difficult to explain, but it could be the result of previous antigenic exposure and the different activation states of the APCs in mice that are housed in different colonies.*"

Doherty expounds on the differences in the types of NKT cells found in humans and mice, with particular emphasis on the types of NKT cells found in the human liver vs. the mouse liver (emphasis added): "Studies in humans have also identified NKT-cell populations that co-express αβ or γδ TCRs and various NK receptors, including CD16, CD56, CD69, CD161, KIR receptors and/or CD94 (46–49). NKT cells account for about one third of all hepatic T cells but about 2% of peripheral T cells (4, 8, 9, 33). *They include a human NK1.1+ T-cell homolog that expresses a TCR that is almost identical to the murine Vα14Jα281 TCR chain, Vα24JαQ (41). However, while Vα14Jα281 TCR expressing NK1.1+ T cells account for about 40% of all hepatic T cells in mice, the human Vα24JαQ TCR is present on a very small percentage of both peripheral and hepatic T cells (8). Therefore, while murine NKT cells represent a homologous population of T cells that express NK1.1 and a Vα14Jα281 TCR, human NKT cells constitute a heterologous population of T cells expressing various TCRs and NK receptors.* Several phenotypic definitions of human NKT cells, based on co-expression of a CD3/TCR complex and either CD16, CD56, CD161 or NK receptors for class I, have been proposed (8, 33). We have found that the co-expression of CD3 and CD56 serves best to define NKT cells that are characteristic of the human liver *(Table 2). This NKT phenotype is most strikingly associated with the liver, and the expression of CD56 by hepatic T cells correlates with the expression of other NK markers and the ability of T cells to mediate NK cytolytic functions. We therefore have defined CD3+CD56+ cells in the human liver as "hepatic natural T (NT) cells" (8). The majority of hepatic NT cells and other NKT cells express the CD8 co-receptor* but CD4+ and DN cells are also present. In our studies of normal donor tissue, *up to 35% of hepatic CD3+CD56+ (NT) cells were found to express γδ TCRs* (4, 8). Ishihara et al. (33) reported that only 10% of hepatic CD3+CD161+ cells express this TCR isotype. However, six of the seven specimens in the latter study were from hepatectomy procedures performed for hepatic malignancy. The presence of a tumor may

significantly influence the local repertoire of T-cell phenotypes. Both we (8) and Ishihara et al. (33) noted that large proportions of hepatic NT and other NKT cells express mature and activated phenotypes in tissue samples from both normal and malignant livers. This suggests that they are actively involved in local immune responses in the liver...A major role for human hepatic NKT cells may be to influence the local immunological milieu through the production of cytokines. In two studies, intracellular staining and flow cytometry were used to detect cytokine production by lymphocyte subsets from normal (9) and hepatitis C virus (HCV)- infected (32) livers. These studies showed that hepatic NT cells, as well as hepatic T cells and NK cells, can produce the inflammatory cytokines IFN-γ and TNF-α upon pharmacological stimulation or CD3 cross-linking (Fig. 7). The inflammatory cytokine IL-2 is also produced by significant proportions of hepatic NT cells and T cells, and a smaller proportion of hepatic NT cells (mean 15%) produce the Th2/Tc2 cytokine IL-4. *As stated above, the predominance of Th1/Tc1 cytokine production by hepatic lymphocytes may reflect the production of proinflammatory cytokines by hepatocytes and Kupffer cells (30, 31) and suggests that the normal liver harbors an inflammatory environment that may be indicative of ongoing immune activity.* IFN-γ and IL-4 can also be produced by murine NK1.1+ T cells (39, 40). *These cells are predominantly IL-4 producers, suggesting that the majority of hepatic NT cells are either functionally distinct from murine NK1.1+ T cells or are polarized by the liver to produce predominantly Th1/Tc1 cytokines* (See Doherty et al., Immunol Rev. 2000 Apr;174:5-20, pages 10-11 and Table 1).

Indeed, it is noted that some of the rejected claims, e.g., claim 32, recite "wherein said NKT cells are NKT cells expressing the CD56 marker." Since NKT cells expressing CD56 are functionally distinct *from murine NK1.1+ T cells or are polarized by the liver to produce predominantly Th1/Tc1 cytokines* as described by Doherty above, the skilled artisan would not know how to use such cells to practice the method as claimed.

With regard to treating any immune-related or immune-mediated disorder with the claimed method, it is noted that the elected species of disease to be treated, "autoimmune liver disease" encompasses in its breadth, for example, the autoimmune liver disease species "autoimmune hepatitis."

As stated by Kaneko et al., J Exp Med. 2000 Jan 3;191(1):105-14, Con A–induced hepatitis is considered to be an experimental murine model of human autoimmune hepatitis (see, in particular, page 106, left column, 1st paragraph). Moreover, as stated by Kaneko, NK1.1+ NKT cells that have been induced to have a high IL-4/IFN-γ expression ratio (see Figure 4) are essential in Con-A induced hepatitis (see Kaneko, Discussion pages 110-112), consistent with the teachings of the instant specification (see, instant specification, for example, page 100, 1ST paragraph).

With regard to using cytokines such as IFNγ or IL12 in step (c) of claim 7 for example, to do so would be antithetical to the claimed method as shown, for example, by Leite-De-Moraes et al., Eur J Immunol. 1998 May;28(5):1507-15, in particular page 1507.

With regard to adding an adhesion molecule such as "integrins, selecins or ICAMs" to the claimed method as in claim 12, to do so would be unpredictable in that expression of the integrin LFA-1 on a liver associated cell is known to be essential for NKT cell homing to the murine liver so adding LFA-1, which could compete with LFA-1 on a liver associated cell for binding to the ex vivo "educated" NKT cells could prevent the administered NKT cells from getting to where they may need to be in order to induce bystander suppression of auto-reactive CD4+ T cells (see Emoto et al., J Immunol. 1999 May 1;162(9):5094-8, in particular, Abstract).

Likewise, the skilled artisan would be hard pressed to predict the effect that the addition of an ICAM, such as ICAM-1 may have on claimed method because based on the teachings of Emoto the skilled artisan would not know with any degree of certainty if addition of ICAM-1 would, for example block the interaction of NKT expressed LFA-1 with liver Kuppfer cells (see Emoto, in particular page 5097, last paragraph).

Likewise, as is well known to the skilled artisan, E-selectin is involved in homing of lymphocytes to the skin, and given that some human Vα24i NKT cells express an E-selectin ligand, it is unclear how ex vivo educated human NKT cells could be used, for example, to treat skin disease after being cultured in the presence of a selectin which could compete with the skin expressed E-selectin for the homing of a subset of ex vivo educated human NKT to the skin where they may need to be in order to induce bystander suppression of auto-reactive CD4+ T cells (see Kim et al., Trends Immunol. 2002 Nov;23(11):516-9, e.g., page 518, right column, 1st paragraph).

Given the heterogeneity of TCR expression, MHC restriction, immune surface marker expression and the absence of substantial knowledge concerning the physiologically relevant allo- or auto- antigen that binds CD1d to activate NKT cells within murine and human NKT cell populations, per se, and the further unpredictability between murine and human NKT cell populations, the skilled artisan would consider predictability in the art of NKT cell based adoptive therapy to be low.

Moreover, the substantial heterogeneity within and between murine and human NKT cells causes it to be nearly impossible for the skilled artisan to begin to understand how to extrapolate from that which is enabled by the instant specification to a method of successfully treating Crohn's disease in a human, much less any immune related or immune mediated disorder or disease in any mammalian subject in need of treatment, by manipulating the NKT cell population to modulate the Th1/Th2 balance toward anti-inflammatory cytokine producing cells resulting in an increase in the quantitative ratio of IL4/IFNγ.

Indeed, the overall uncertainty in the art of NKT cells is nicely summarized in the review of Kronenberg which states at page 564, right column, 1st paragraph (emphasis added), "TABLE 3 summarizes the diverse types of immune response and pathological condition for which there is evidence that Vα14*i* and/or Vα24*i* T cells influence the outcome. The information is not definitive, and there are likely to be continuing controversies about the

importance of Vα14*i* T cells in different contexts… *The collective findings highlight the diverse effector mechanisms that are initiated by Vα14i T-cell stimulation, and the almost unpredictable outcomes that are obtained when studying these lymphocytes. Therefore, just as the global, adaptive immune response could be pushed unpredictably in either a TH2 or TH1 direction after Vα14i T-cell activation*, the influence of Vα14*i* T cells in different conditions can be attributed to either TH2 cytokines (such as IL-4 or IL-13), TH1 cytokines, cytotoxicity or regulatory cytokines, such as IL-10."

In view of the quantity of experimentation necessary, the limited working examples, the unpredictability of the art, the lack of sufficient guidance in the specification, and the breadth of the claims, undue experimentation would be required to produce the claimed invention commensurate with the scope of the claims.

The scope of the claims must bear a reasonable correlation with the scope of enablement. *In re Fisher*, 166 USPQ 18(CCPA 1970) indicates that the more unpredictable an area is, the more specific enablement is necessary in order to satisfy the statute.

The issue is make and use, not attempt to make and then test to see if the skilled artisan could use. The specification does not enable the genus because where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. In re Soll, 97 F.2d 623, 624, 38 USPQ 189, 191 (CCPA 1938). In cases involving unpredictable factors, such as most chemical reactions and physiological activity, more may be required. In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970) (contrasting mechanical and electrical elements with chemical reactions and physiological activity). This is because it is not obvious from the disclosure of one particular species, what other species will work, and if little is known in the prior art about the nature of the claimed invention, i.e., treating immune-related or immune-mediated disorders or diseases in a mammalian subject in need of such treatment, by manipulating the NKT cell population of said subject, wherein manipulation of said NKT cell population results in modulation of the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, said modulation being mediated by different components, cells, tissues or organs of said subject's or another subject's immune system comprising the steps of: a. obtaining NKT cells from said subject or another subject; b. *ex vivo* educating the NKT cells obtained in step (a) such that the resulting educated NKT cells may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells; and c. re-introducing to said subject the educated NKT cells obtained in step (b) which may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ratio between any one of IL4 and IL 10 to IFNγ, wherein said *ex vivo* education of step (b) is performed by culturing said NKT cells in the presence of any one of: a. antigens or epitopes associated with said immune-related or immune-mediated disorder or disease to be treated, antigens or epitopes associated with the immune-mediated inflammatory response, or any combination thereof; b. at least one liver-associated cell of tolerized or non-tolerized subjects suffering from said immune-related or immune-mediated disorder or of said subject; c. at least one cytokine or adhesion molecule, or any combination thereof; and d. a combination of any of (a), (b) and

(c), and wherein said NKT cells may optionally express the CD56 marker, and the art is unpredictable, as in the instant case, the specification would need more detail as to how to make and use the invention in order to be enabling. See MPEP 2164.03.

Further, as stated in Rasmusson v. SmithKline Beecham Corp., 75 USPQ2d 1297-1303 (CAFC 2005), "If mere plausibility were the test for enablement under section 112, applicants could obtain patent rights to 'inventions' consisting of little more than respectable guesses as to the likelihood of their success. When one of the guesses later proved true, the 'inventor' would be rewarded the spoils instead of the party who demonstrated that the method actually worked. That scenario is not consistent with the statutory requirement that the inventor enable an invention rather than merely proposing an unproved hypothesis."

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –
(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 2, 3, 6-13, 15, 19, 32, 144-151, 165 and 166 are rejected under 35 U.S.C. 102(b) as being anticipated by Ilan Yaron (WO 02051986, cited on an IDS).

Ilan Yaron teaches the claimed invention, in particular a method of treating Crohn's disease according to the claimed invention, see Examples 1 and 3-7 on pages 48-66 and claims 1-2, 4-15, 17, 18, 43 and 44.

Thus, Ilan Yaron anticipates the instant claims.

7. No claim is allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ZACHARY SKELDING whose telephone number is (571)272-9033. The examiner can normally be reached on Monday - Friday 8:00 a.m. - 5:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Eileen O'Hara can be reached on 571-272-0878. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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August 8, 2008

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